**Supplemental Methods, Figures and References**

**Supplementary Materials and Methods**

*Antimicrobial Treatment*

Mice (5 *Tff1*-WT and 5 *Tff1*-KO) were housed in individually ventilated cages supplied with autoclaved filtered water and rodent chow. Antibiotic treatment started at 3 weeks of age, immediately after weaning. Mice were given autoclaved drinking water containing 0.68 mg/mL metronidazole and 0.34 mg/mL ciprofloxacin until the age of 12 weeks (1). Upon completion of the treatment, mice received autoclaved water and rodent chow and were handled in sterile conditions for 12 weeks. The mice were sacrificed and stomach tissues were collected for histological evaluation.

*Treatment with Celecoxib*

At the age of three weeks, immediately after weaning, twenty *Tff1*-knockout mice were divided into two equal groups of ten each; one control and one experimental group. The control group received intra peritoneal injection of vehicle (5μl/g body weight). The vehicle consisted of propylene glycerol, polysorbate 80, benzyl alcohol, ethanol and water (40:0.5:1:10:48.5) (2). The experimental group received Celecoxib (10μg/g). Injections were given three times a week for two months. Animals were sacrificed and stomach tissues were collected for histological evaluation.

*Rescue of Tff1 effects/signaling in Primary Gastric Epithelial Cells*

Because TFF1 is a secreted protein, we decided to confirm the role of TFF1 in regulating NF-kB ex-vivo in primary gastric epithelial cell cultures. We performed a rescue experiment using conditioned media from AGS-pcDNA and AGS-TFF1 cells.  Primary gastric epithelial cells from pyloric antral region of *Tff1*-knockout mice were cultured in three parallel 8-well chambers for 24 hours. On the next day, cells were washed with PBS and media was replaced with either conditioned media from AGS-pcDNA (chamber A) or AGS-TFF1 cell lines culture (chambers B and C). After another 48 hours, cells in chambers A and B were processed for dual immunofluorescence staining with rabbit anti-NF-kB p65 antibody (Gene Script)

and mouse anti-Zo1 antibody (ZYMED Laboratories, Invitrogen). Cells treated with AGS-TFF1 conditioned media in chamber C were washed with PBS and treated with AGS-pcDNA conditioned media. Following an additional 24 hours, these cells were processed for dual immunofluorescence staining as above. All experiments were performed in triplicates.

**Supplemental Figure Legends**

**Figure S1.**

**A**: Representative images of stomachs from *Tff1*-KO mice at 8 months of age and older showing large tumors in the antrum region. **B**: Ki-67 immunostaining (brown nuclei) in WT mice reveals dividing cells in the proliferative zone of the mucosa (left panel); in *Tff1*-KO, stained cells extended to the surface (right panel); original magnification, x10. **C&D:** Box plots representing the comparison of chronic and acute inflammation scores between *Tff1*-WT and *Tff1*-KO mice. **E&F**: Box plots representing the comparison of chronic and acute inflammation scores between male and female *Tff1*-KO mice, respectively. **G**: Comparison of Acute inflammation scores between different dysplastic lesions. **H**: Comparison of chronic inflammation scores between *Tff1*-WT and *Tff1*-KO treated with Ciprofloxacin and Metronidazole. Box-and-whisker plots were used depict the smallest value, lower quartile, mean, upper quartile, and largest value.

**Figure S2. Morphological histology of different tissues from the same *Tff1*-KO mice**

**A-F:** Hematoxylin & eosin (H&E) staining of representative histological features of gastrointestinal track (Stomach, Esophagus, and Colon) and other organs (Liver, Spleen and Thymus) from the same *Tff1*-knockout mouse. **A:** antro-pyloric area showing dysplasia (square) and infiltration of inflammatory cells (circle); original magnification x4. The inset in panel A displays a higher magnification (x40) of dysplastic glands. **B-F:** All other tissues appear to be normal; original magnification, x10 (B, D, E & F) and x20 (C).

**Figure S3.**

**A-I:** Validation of selected inflammatory genes from microarray results. Relative mRNA expression levels were examined in the antral gastric tissues from wild-type and *Tff1*-knockout mice at different ages. Horizontal bars indicate the mean and *p≤0.05* was considered statistically significant.

**Figure S4. Celecoxib treatment affects the development of gastric lesions in the *Tff1*-KO mice**

**A:** Bars representing the comparison of frequency of dysplasia between vehicle (n=10) and celecoxib (n=10) treated *Tff1*-KO mice. **B&C:** qRT-PCR showing a decrease in mRNA expression of pro-inflammatory genes (*Cxcl1 and Cxcl5)* in celecoxib-treated group normalized to Vehicle.

**Figure S5: Inverse correlation between TFF1 and pIKKα/β expression**

**A:** qRT-PCR representing the human *TFF1* gene expression in AGS TFF1 clone 1 & 2 normalized to AGS-pcDNA. **B:** Western Blot analysis showing the expression of pIKKα/β and total IKKβ in AGS-pcDNA and AGS-TFF1 clone 1.

**Figure S6: Expression of *Tff1* in mouse tissues**

qRT-PCR of *Tff1* in tissues from *Tff1*-wild-type mice showing high expression levels in the pyloro-antral regions of the stomach. In contrast, *Tff1* expression was almost undetectable in different immune system organs: Thymus, Bone marrow (BM) and spleen.
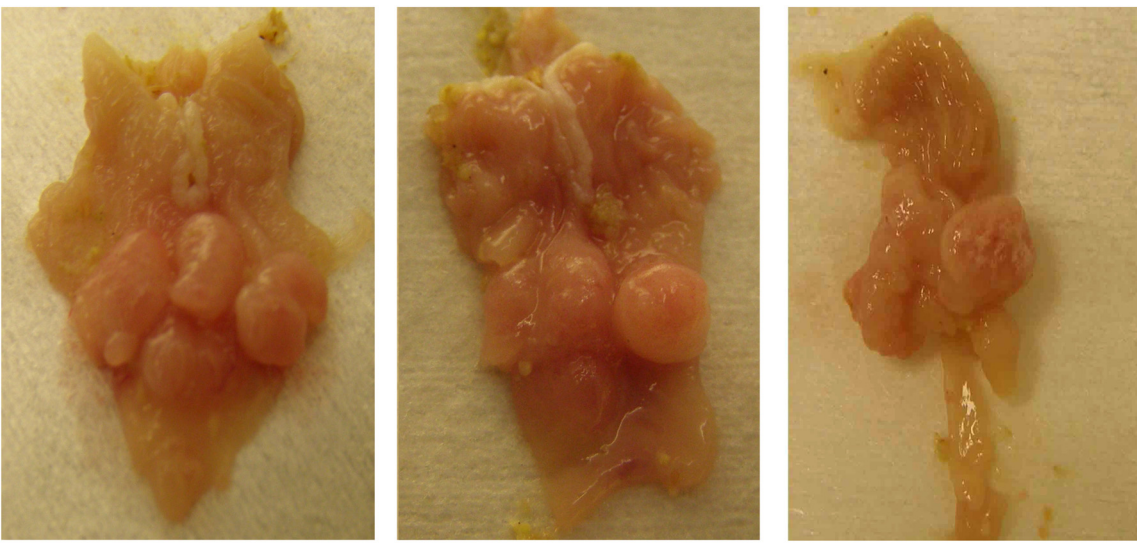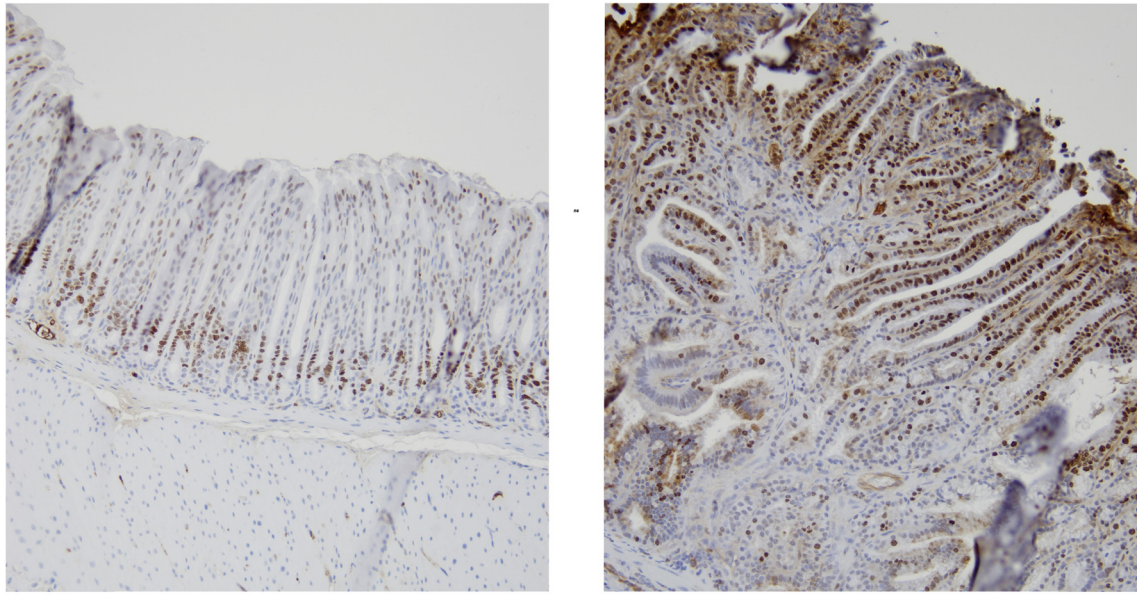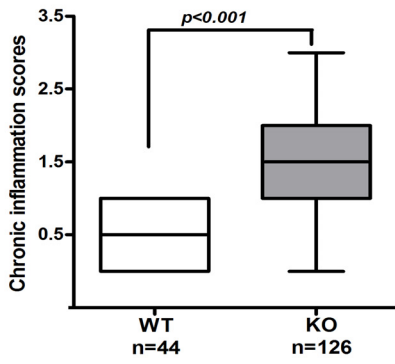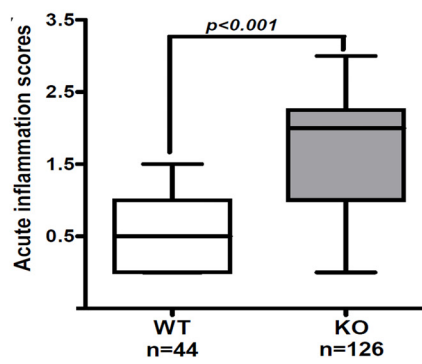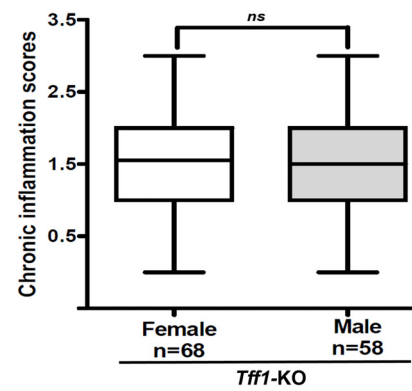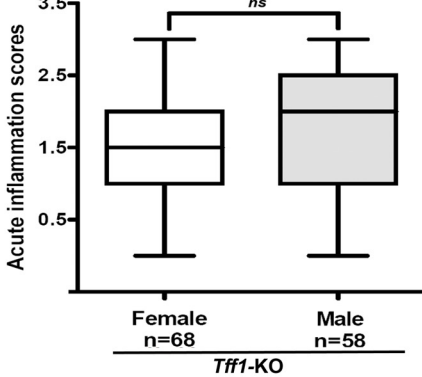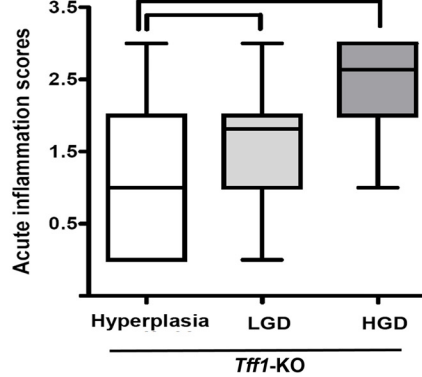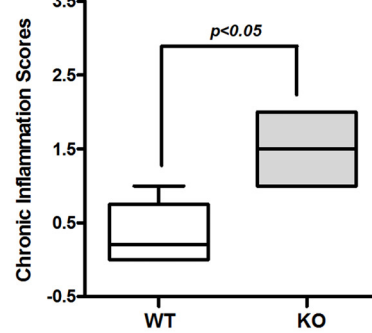
**Figure S7: Expression of *TNFR1* and *TNFR2*.**

**A:** qRT-PCR demonstrated high levels of *TNFR1* in AGS-pcDNA and AGS-TFF1 clones 1 and 2 whereas

*TNFR2* levels were almost undetectable in the same cells. **B:** qRT-PCR showing similar levels of mRNA

expression of *Tnfr1* in the stomach of *Tff1*-knockout and *Tff1*-wild type mice.

**Figure S8: Downregulation of *TFF1* mRNA in human gastric cancers**

**A:** qRT-PCR analysis of 70 human gastric adenocarcinomas and 36 normal gastric epithelial tissues

demonstrated a significant reduction in *TFF1* mRNA expression in tumor samples (p<0.001). **B:** qRT-PCR

analysis of 15 matched gastric cancer (tumor) and adjacent non-tumor (normal) samples indicated that

the *TFF1* expression was significantly downregulated in the tumor samples as compared to their

adjacent histologically normal tissues (P<0.01).

**SUPPLEMNTAL REFERNECES**

1.      Judd, L.M., Bredin, K., Kalantzis, A., Jenkins, B.J., Ernst, M., and Giraud, A.S. 2006. STAT3

        activation regulates growth, inflammation, and vascularization in a mouse model of gastric

        tumorigenesis. *Gastroenterology* 131:1073-1085.

2.      Zheng, X., Cui, X.X., Avila, G.E., Huang, M.T., Liu, Y., Patel, J., Kong, A.N., Paulino, R., Shih, W.J.,

        Lin, Y., et al. 2007. Atorvastatin and celecoxib inhibit prostate PC-3 tumors in immunodeficient

        mice. *Clin Cancer Res* 13:5480-5487.

Figure S1. Soutto et al.

**Stomach**  **Esophagus**  **Colon**

A  B  C

**Liver**  **Spleen**  **Thymus**

D  E  F
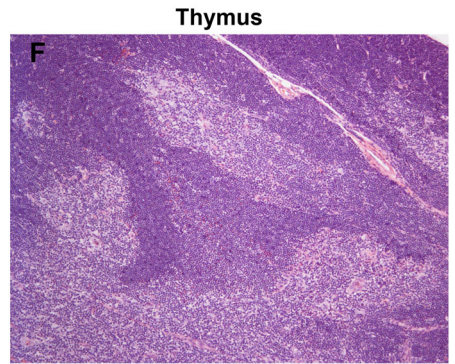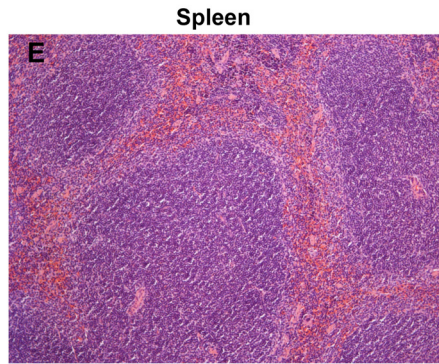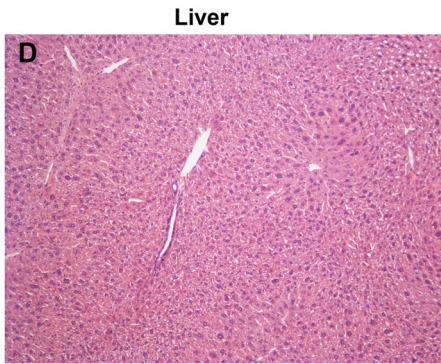
**Figure S2. Soutto et al.**

Figure S3. Soutto et al.

**A**

Frequency of dysplasia (%)

*p=0.03*

9/10

4/10

Vehicle    Celecoxib

**Figure S4, Soutto et al.**

**B**

*Cxcl1*

Fold mRNA expression

*p<0.05*

Vehicle    Celecoxib

**C**

*Cxcl5*

Fold mRNA expression

*p<0.001*

Vehicle    Celecoxib

**A**

Relative fold mRNA expression (y-axis, log scale: 10 to 100000)

x-axis: pcDNA, C-1, C-2 (TFF1)

**B**

pIKKα/β
(Ser 176/180)

Total IKKβ

pcDNA    TFF1 C1

**Figure S5. Soutto et al.**

**Figure S6. Soutto et al.**

**A**



**B**

*Tnfr1*

Figure S7. Soutto et al.

**A**

**Fold mRNA expression**

Normal
n=36

Tumor
n=70

*p<0.001*

**B**

**Fold mRNA expression**

Normal
n=15

Tumor
n=15

*p<0.01*

**Figure S8. Soutto et al.**

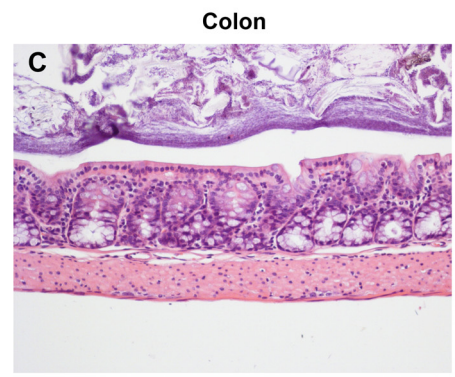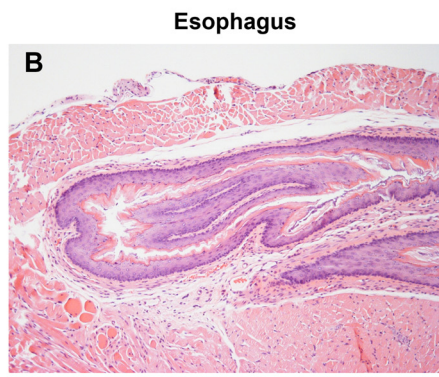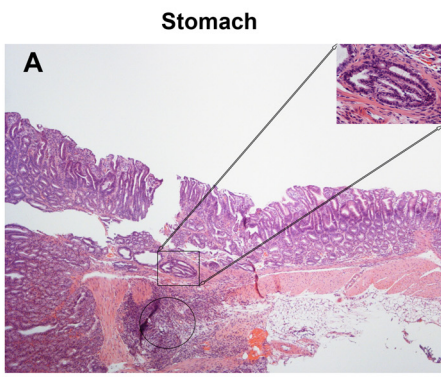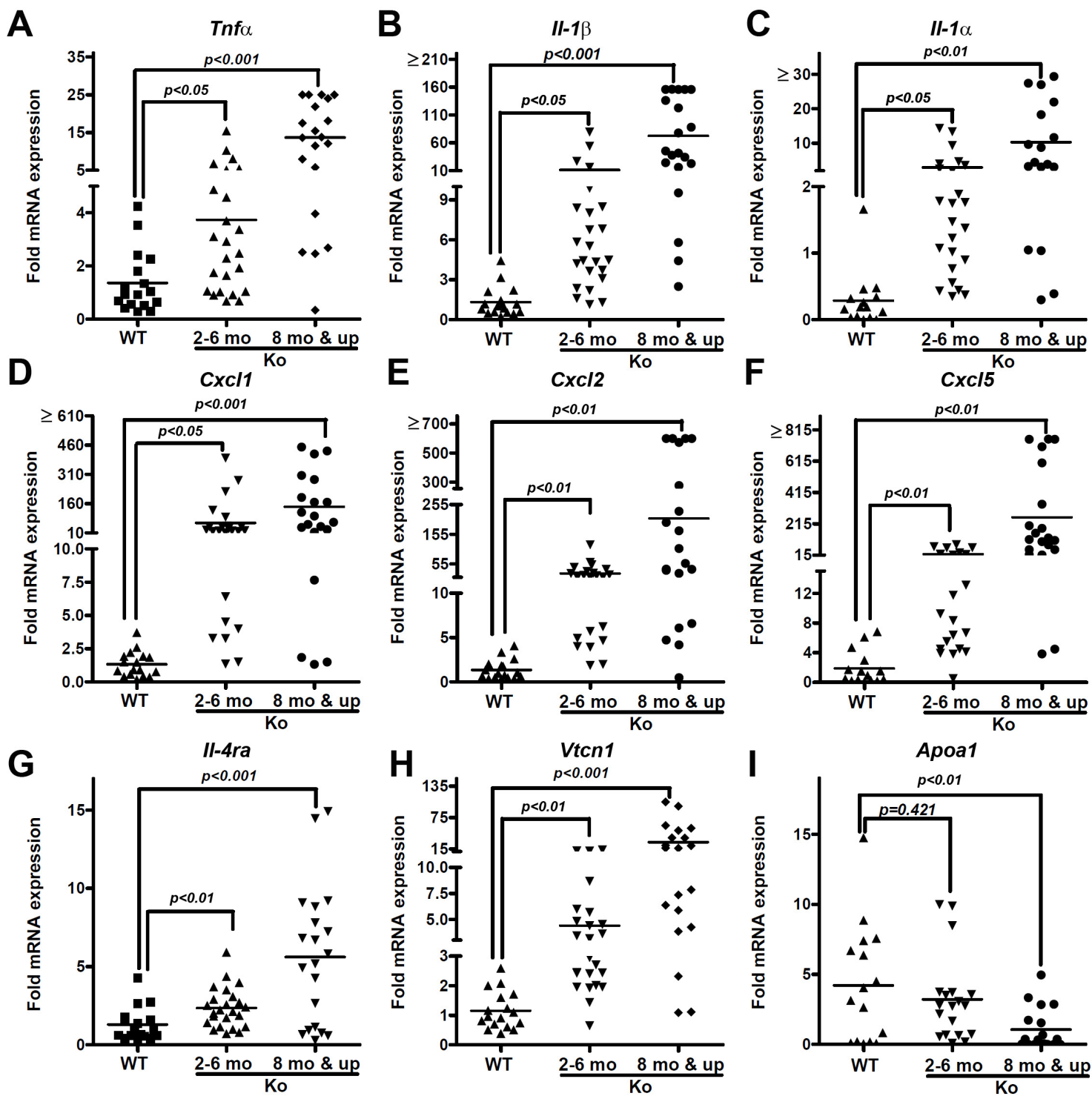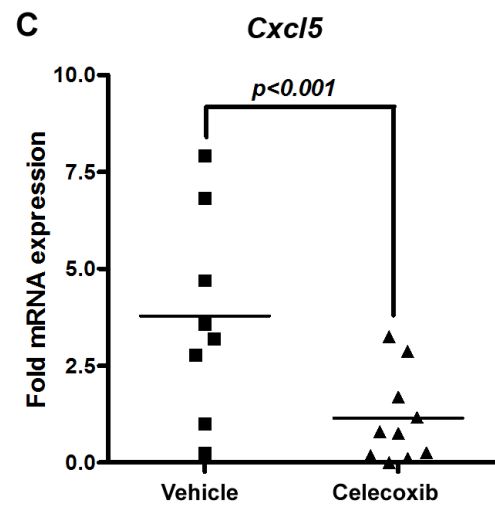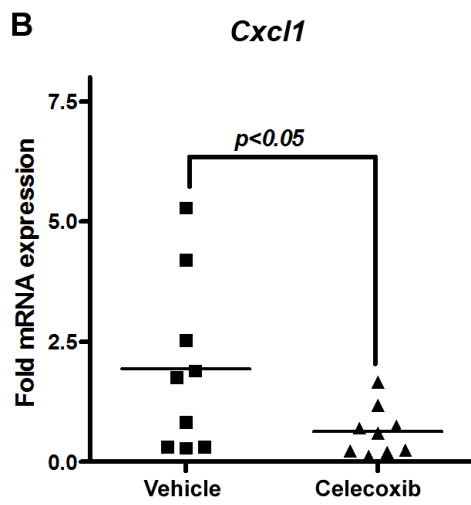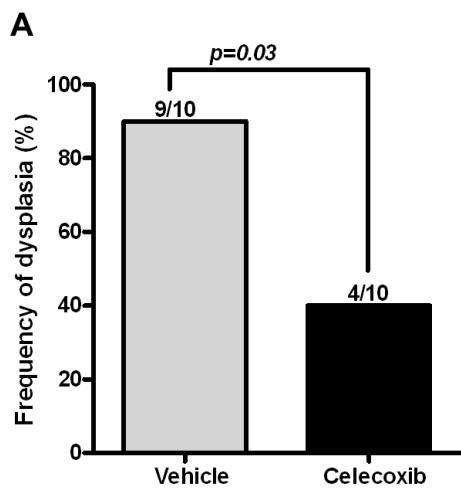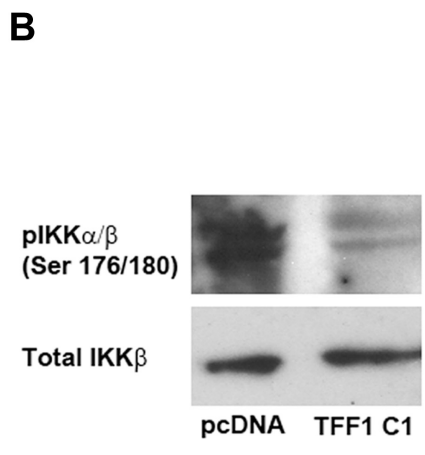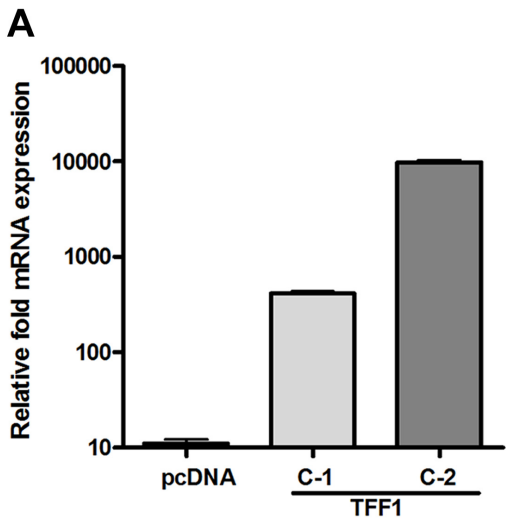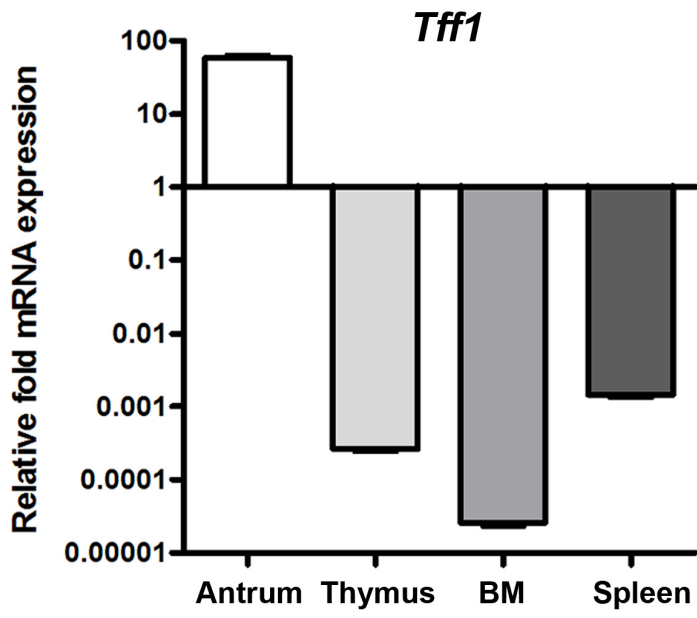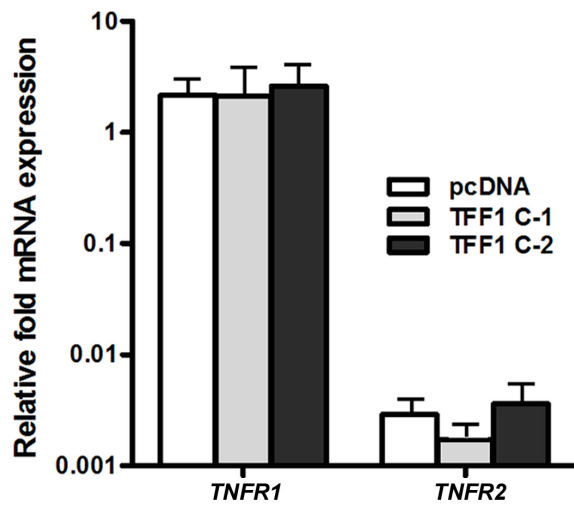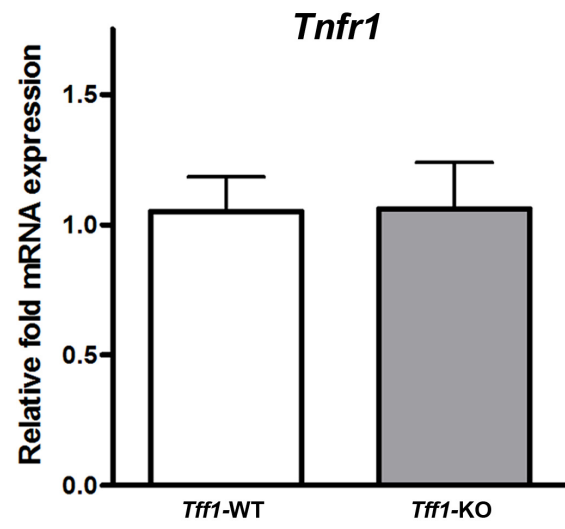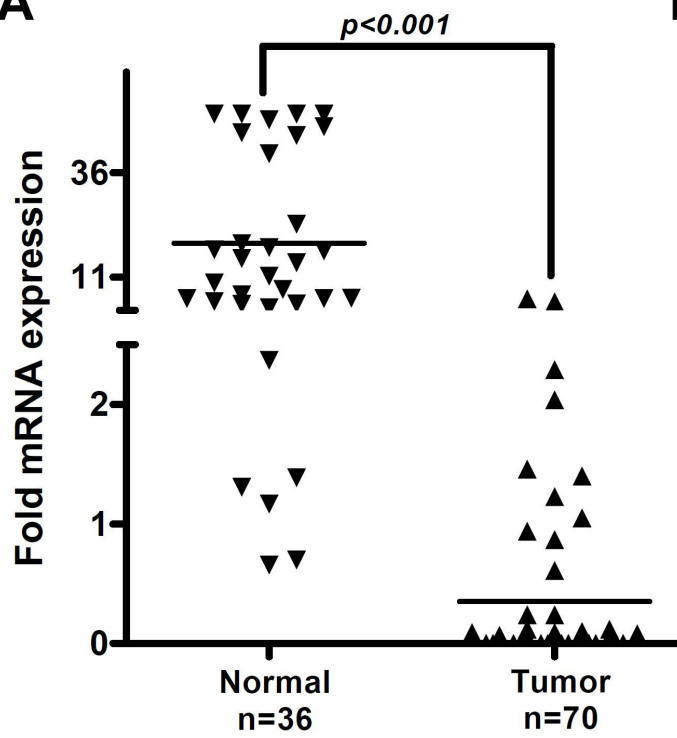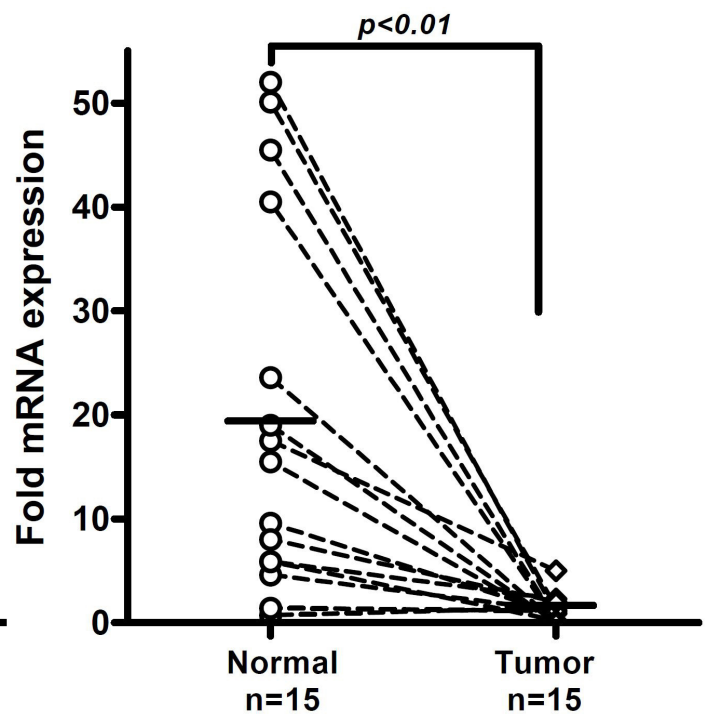**Supplemental Table 1. Fold change of inflammation-related genes in Tff1 KO vs WT**

| ID | Symbol | UGCluster | Fold (KO vs WT) | PValue | Chromsome Cytoband | Name |
|---|---|---|---|---|---|---|
| 1450105_at | Adam10 | Mm.3037 | 3.3 | 2.E-03 | 9 D | a disintegrin and metallopeptidase domain 10 |
| 1426619_at | Aim2 | Mm.131453 | 3.3 | 2.E-03 | 1 H3 | absent in melanoma 2 |
| 1419091_a_at | Anxa2 | Mm.238343 | 2.9 | 7.E-04 | 9 C | annexin A2 |
| 1438840_x_at | Apoa1 | Mm.26743 | 0.0 | 5.E-08 | 9 A2-A4 | apolipoprotein A-I |
| 1417761_at | Apoa4 | Mm.4533 | 0.1 | 4.E-07 | 9 A5.2 | apolipoprotein A-IV |
| 1417776_at | Azgp1 | Mm.30061 | 0.2 | 3.E-03 | 5 G2 | alpha-2-glycoprotein 1, zinc |
| 1422084_at | Bmx | Mm.504 | 3.0 | 2.E-02 | X F | BMX non-receptor tyrosine kinase |
| 1418021_at | C4b | Mm.439678 | 4.4 | 6.E-03 | 17 B1 | complement component 4B (Childo blood group) |
| 1426165_a_at | Casp3 | Mm.34405 | 3.1 | 4.E-03 | 8 B1.1 | caspase 3 |
| 1417268_at | Cd14 | Mm.3460 | 5.6 | 3.E-04 | 18 B2 | CD14 antigen |
| 1450884_at | Cd36 | Mm.18628 | 0.0 | 5.E-06 | 5 A3 | CD36 antigen |
| 1423760_at | Cd44 | Mm.423621 | 8.5 | 6.E-04 | 2 E2 | CD44 antigen |
| 1441907_s_at | Cd93 | Mm.681 | 5.8 | 4.E-05 | 2 G3 | CD93 antigen |
| 1452532_x_at | Ceacam1 | Mm.322502 | 6.2 | 4.E-04 | 7 A3 | carcinoembryonic antigen-related cell adhesion molecule 1 |
| 1427767_a_at | Cftr | Mm.15621 | 7.6 | 1.E-02 | 6 3.1 cM | cystic fibrosis transmembrane conductance regulator homolog |
| 1424495_a_at | Cklf | Mm.269219 | 3.2 | 8.E-05 | 8 D3 | chemokine-like factor |
| 1416953_at | Ctgf | Mm.390287 | 4.0 | 1.E-03 | 10 A3-B1 | connective tissue growth factor |
| 1457644_s_at | Cxcl1 | Mm.21013 | 18.0 | 9.E-03 | 5 E-F | chemokine (C-X-C motif) ligand 1 |
| 1449984_at | Cxcl2 | Mm.4979 | 14.4 | 1.E-02 | 5 E1 | chemokine (C-X-C motif) ligand 2 |
| 1438148_at | Cxcl3 | Mm.244289 | 8.6 | 3.E-02 | 5 E1 | chemokine (C-X-C motif) ligand 3 |
| 1419728_at | Cxcl5 | Mm.4660 | 6.3 | 8.E-03 | 5 E1 | chemokine (C-X-C motif) ligand 5 |
| 1454268_a_at | Cyba | Mm.271671 | 3.1 | 3.E-04 | | cytochrome b-245, alpha polypeptide |
| 1418287_a_at | Dmbt1 | Mm.4138 | 5.1 | 2.E-04 | 7 F4 | deleted in malignant brain tumors 1 |
| 1429021_at | Epha4 | Mm.400747 | 4.1 | 2.E-04 | 1 C1-C5 | Eph receptor A4 |
| 1417408_at | F3 | Mm.273188 | 4.3 | 1.E-02 | 3 G1 | coagulation factor III |
| 1449269_at | F5 | Mm.12900 | 5.8 | 7.E-03 | 1 H2.2 | coagulation factor V |
| 1423100_at | Fos | Mm.246513 | 3.4 | 3.E-04 | 12 D2 | FBJ osteosarcoma oncogene |
| 1422555_s_at | Gna13 | Mm.193925 | 3.0 | 6.E-03 | 11 E1 | guanine nucleotide binding protein, alpha 13 |
| 1425357_a_at | Grem1 | Mm.166318 | 4.4 | 2.E-02 | 2 E4 | gremlin 1 |
| 1418350_at | Hbegf | Mm.289681 | 3.9 | 1.E-03 | 18 B2 | heparin-binding EGF-like growth factor |
| 1427127_x_at | Hspa1b | Mm.372314 | 3.0 | 3.E-02 | 17 B1 | heat shock protein 1B |

| 1419647_a_at | Ier3 | Mm.25613 | 3.6 | 6.E-04 | 17 B1 | immediate early response 3 |
|---|---|---|---|---|---|---|
| 1449982_at | Il11 | Mm.35814 | 3.8 | 3.E-02 | 7 A1 | interleukin 11 |
| 1451775_s_at | Il13ra1 | Mm.24208 | 5.4 | 5.E-04 | X A3.3 | interleukin 13 receptor, alpha 1 |
| 1421628_at | Il18r1 | Mm.253664 | 3.4 | 2.E-03 | 1 B | interleukin 18 receptor 1 |
| 1421291_at | Il18rap | Mm.20466 | 3.5 | 1.E-02 | 1 B | interleukin 18 receptor accessory protein |
| 1449399_a_at | Il1b | Mm.222830 | 7.6 | 2.E-02 | 2 F | interleukin 1 beta |
| 1448950_at | Il1r1 | Mm.896 | 4.1 | 7.E-05 | 1 B | interleukin 1 receptor, type I |
| 1422317_a_at | Il1rl1 | Mm.289824 | 7.9 | 2.E-02 | 1 B | interleukin 1 receptor-like 1 |
| 1451798_at | Il1rn | Mm.882 | 11.7 | 3.E-04 | 2 A3 | interleukin 1 receptor antagonist |
| 1421034_a_at | Il4ra | Mm.233802 | 2.9 | 8.E-04 | 7 F3 | interleukin 4 receptor, alpha |
| 1422053_at | Inhba | Mm.8042 | 9.3 | 4.E-03 | 13 A1 | inhibin beta-A |
| 1450501_at | Itga2 | Mm.5007 | 4.1 | 2.E-03 | 13 D2.2 | integrin alpha 2 |
| 1451336_at | Lgals4 | Mm.210336 | 3.5 | 9.E-04 | 7 A3 | lectin, galactose binding, soluble 4 |
| 1416304_at | Litaf | Mm.294753 | 4.4 | 5.E-05 | 16 B1-B3 | LPS-induced TN factor |
| 1422352_at | Mcpt1 | Mm.201549 | 11.4 | 2.E-03 | 14 C3 | mast cell protease 1 |
| 1422990_at | Met | Mm.86844 | 2.9 | 1.E-03 | 6 4.0 cM | met proto-oncogene |
| 1417256_at | Mmp13 | Mm.5022 | 14.3 | 8.E-03 | 9 A1-A2 | matrix metallopeptidase 13 |
| 1418945_at | Mmp3 | Mm.4993 | 6.7 | 7.E-03 | 9 A1 | matrix metallopeptidase 3 |
| 1419127_at | Npy | In multiple Geneic | 3.1 | 1.E-02 | | |
| 1419123_a_at | Pdgfc | Mm.331089 | 3.2 | 2.E-03 | 3 E3 | platelet-derived growth factor, C polypeptide |
| 1450060_at | Pigr | Mm.276414 | 6.8 | 2.E-05 | 1 E3 | polymeric immunoglobulin receptor |
| 1415806_at | Plat | Mm.154660 | 3.0 | 1.E-02 | 8 A2 | plasminogen activator, tissue |
| 1452521_a_at | Plaur | Mm.1359 | 4.8 | 3.E-03 | 7 A3 | plasminogen activator, urokinase receptor |
| 1457088_at | Pldn | Mm.268921 | 4.2 | 2.E-03 | 2 E5 | pallidin |
| 1429527_a_at | Plscr1 | Mm.421956 | 3.4 | 4.E-04 | 9 E3.3 | phospholipid scramblase 1 |
| 1416957_at | Pou2af1 | Mm.897 | 5.8 | 7.E-04 | 9 A5.3 | POU domain, class 2, associating factor 1 |
| 1421073_a_at | Ptger4 | Mm.18509 | 9.0 | 9.E-06 | 15 A1 | prostaglandin E receptor 4 (subtype EP4) |
| 1417262_at | Ptgs2 | Mm.292547 | 7.0 | 4.E-02 | 1 H1 | prostaglandin-endoperoxide synthase 2 |
| 1420710_at | Rel | Mm.4869 | 3.0 | 2.E-02 | 11 A3.2 | reticuloendotheliosis oncogene |
| 1426604_at | Rnasel | Mm.259254 | 4.2 | 5.E-04 | 1 G2 | ribonuclease L (2', 5'-oligoisoadenylate synthetase-dependent) |
| 1419394_s_at | S100a8 | Mm.21567 | 6.3 | 4.E-02 | 3 F1-F2 | S100 calcium binding protein A8 (calgranulin A) |
| 1419480_at | Sell | Mm.1461 | 3.3 | 3.E-02 | 1 H2.2 | selectin, lymphocyte |
| 1420558_at | Selp | Mm.3337 | 3.9 | 2.E-02 | 1 H2.2 | selectin, platelet |
| 1420378_at | Sftpd | Mm.1321 | 9.5 | 5.E-05 | 14 B | surfactant associated protein D |
| 1455899_x_at | Socs3 | Mm.3468 | 5.0 | 4.E-03 | 11 E2 | suppressor of cytokine signaling 3 |

| 1460302_at | Thbs1 | Mm.4159 | 3.5 | 4.E-02 | 2 F1-F3 | thrombospondin 1 |
|---|---|---|---|---|---|---|
| 1418685_at | Tirap | Mm.23987 | 3.7 | 2.E-05 | 9 A5.3 | toll-interleukin 1 receptor (TIR) domain-containing adaptor protein |
| 1449049_at | Tlr1 | Mm.273024 | 3.4 | 6.E-03 | 5 C3.1 | toll-like receptor 1 |
| 1419132_at | Tlr2 | Mm.87596 | 3.0 | 1.E-03 | 3 E3 | toll-like receptor 2 |
| 1442827_at | Tlr4 | Mm.38049 | 2.8 | 2.E-03 | 4 C1 | toll-like receptor 4 |
| 1418571_at | Tnfrsf12a | Mm.28518 | 3.6 | 9.E-04 | 17 A3.3 | tumor necrosis factor receptor superfamily, member 12a |
| 1419083_at | Tnfsf11 | Mm.249221 | 2.9 | 3.E-02 | 14 D3 | tumor necrosis factor (ligand) superfamily, member 11 |
| 1447845_s_at | Vnn1 | Mm.27154 | 21.7 | 3.E-05 | 10 A1-B2 | vanin 1 |
| 1458070_at | Vtcn1 | Mm.137467 | 5.8 | 1.E-05 | 3 F2.2 | V-set domain containing T cell activation inhibitor 1 |
| 1455098_a_at | Vtn | Mm.3667 | 0.1 | 5.E-05 | 11 B5 | vitronectin |

**Supplemental Table 2.**

**Mouse primer sequences used in quantitative real-time RT-PCR**

| Gene | RefSeq No. | Forward primer | Reverse primer | Product Size (bp) |
|------|-----------|----------------|----------------|-------------------|
| Tff1 | NM_009362.2 | CCCGGGAGAGGATAAATTGT | GCCAGTTCTCTCAGGATGGA | 173 |
| Tnfα | NM_013693.2 | CCACCACGCTCTTCTGTCTA | AGGGTCTGGGCCATAGAACT | 100 |
| Il1β | NM_008361.3 | CTTTGAAGAAGAGCCCATCC | CATCTCGGAGCCTGTAGTGC | 101 |
| Il1α | NM_010554.4 | TCAAGATGGCCAAAGTTCCT | TGAGCCATAGCTTGCATCAT | 131 |
| Cxcl1 | NM_008176.3 | GCTGGGATTCACCTCAAGAA | TCTCCGTTACTTGGGGACAC | 180 |
| Cxcl2 | NM_009140.2 | AGTGAACTGCGCTGTCAATG | TTCAGGGTCAAGGCAAACTT | 153 |
| Cxcl5 | NM_009141.2 | GCCCTACGGTGGAAGTCATA | GTGCATTCCGCTTAGCTTTC | 132 |
| Il4rα | NM_001008700.3 | TTTTTGAAGTTGCTGGAGAGG | CGTGGAAGTGCGGATGTAGT | 100 |
| Vtcn1 | NM_178594.3 | CCTCTCCATGGCTTCCTTG | GAAATGCCAAAGCCAATGAT | 102 |
| Apoa1 | NM_009692.3 | GCCAACAGCTGAACCTGAAT | CAGAAGTCCCGAGTCAATGG | 100 |

**Human primer sequences used in quantitative real-time RT-PCR**

| Gene | RefSeq No. | Forward primer | Reverse primer | Product Size (bp) |
|------|-----------|----------------|----------------|-------------------|
| TFF1 | NM_003225.2 | GGTCCTGGTGTCCATGCTG | ACAGCAGCCCTTATTTGCAC | 136 |
| CXCL5 | NM_002994.3 | GATCCAGAAGCCCCTTTTCT | GAAACTTTTCCATGCGTGCT | 101 |
| IL-4Rα | NM_000418.2 | GGCGCGCAGATAATTAAAGA | GCTGGTCTCGAACTCCTGAT | 139 |
| TIRAP | NM_148910.2 | CAGCCCTCATCGCAACTG | TTCTAGTGACACTTCTCCTGTGAAA | 101 |
| BIRC3 | NM_001165.3 | TTAACTGGCCCTCTAGTGTTCT | CACCATCACAGCAAAAGCAT | 105 |
| SMAC | NM_019887.4 | TCATAGGAGCCAGAGCTGAGA | TTCTGCTGCCATCTCTGAAA | 101 |

**Supplemental Table 3. Calculation of the IHC composite expression score (CES)**

| | | Frequency | | |
| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| **0** | 0 | 0 | 0 | 0 |
| **1** | 1 | 2 | 3 | 4 |
| **2** | 5 | 6 | 7 | 8 |
| **3** | 9 | 10 | 11 | 12 |

(Intensity is the row label on the left)

To obtain an IHC continuos score that takes into account the IHC signal intensity and the frequency of positive cells, we generated a composite expression score (CES) with full range from 0 to 12. The CES is calculated using the formula; CES = 4(intensity-1)+frequency.